Angew. Botanik 61, 157–162 (1987)
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ISSN 0066-1759/ASTM-Coden: ANBTAJ

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Growth of Mycorrhizal Fungi Cultured with Bacteria

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Summary

Cultures of 4 different mycorrhizal fungi of pine (*Pinus sylvestris* L.) were grown at 20 °C at pH 4.0, 5.0 or 6.0 with each of 5 bacterial strains isolated from the mycorrhizosphere of pine.

In media inoculated simultaneously with the fungi and bacteria, the growth of the fungi was in most cases slightly stimulated. When bacteria were introduced into 7 days old cultures of the fungi stimulation of growth of the fungi was noted.

Introduction

The saprophytic microorganisms of the soil and rhizosphere may have a favourable or detrimental effect on growth of plants and development of pathogenic and symbiotic organisms in the root zone (Katznelson, 1965; Bowen and Theodorou, 1979; Baker, 1980).

The importance of soil organisms in colonization of the rhizosphere by ectomycorrhizal fungi has been stressed by Levisohn (1957), Rambelli (1973) and Bowen and Theodorou (1979).

Also environmental factors such as pH, temperature and presence of nutritional and growth substances may affect the mycorrhizal fungi (Hacskaylo, 1973; Slankis, 1973). Even intraspecific differences in pH requirement in some mycorrhizal fungi isolates were noted (Hung and Trappe, 1983).

The effect of environmental factors on mycorrhizal fungi is certainly different in monoxenic and dixenic cultures. Therefore this study was undertaken.

Materials and Methods

The following ectomycorrhizal fungi of pine: *Amanita muscaria, Suillus bovinus, S. luteus, Rhizopogon luteolus* and 5 bacterial strains isolated from the mycorrhizosphere of pine were used in our study. The bacterial strains were pleomorphic types identical or similar to *Arthrobacter* sp.

The fungal isolates were obtained from the Institute of Dendrology, Polish Academy of Sciences, Kórnik, Poland.

The fungi were grown in liquid Melin and Rama Das (1954) medium adjusted to pH 4.0, 5.0 or 6.0 at 20 °C.

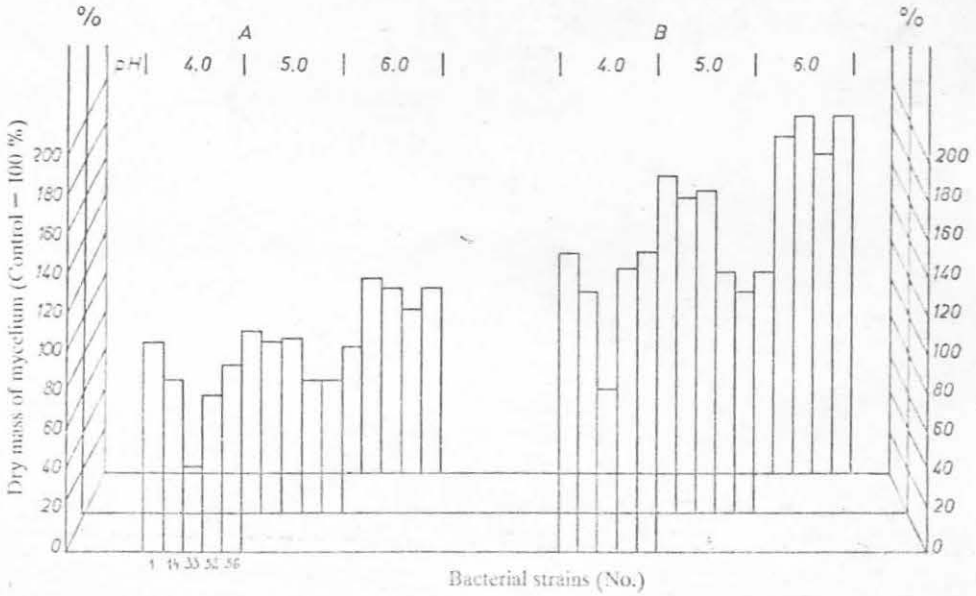
Erlenmeyer flasks (300 ml capacity) containing 100 ml of the above medium were inoculated with two discs (1 cm in diameter) of a 14-day-old fungal culture grown on the same agar medium and 0.5 ml of bacterial suspension obtained by washing off 3-day-old slants prepared from medium according to Lochhead and Chase (1943).

The inoculations of bacteria and fungi were done either simultaneously or 7-day-old cultures of the fungi were seeded with bacteria. After 21 days of growth the mycelium was separated from the medium by filtration on filter paper and the dry mass was determined.

Results

The results obtained in this work are presented on Figures 1—4. In media inoculated simultaneously with the fungus and bacteria no harmful effect on growth of the fungus was observed or in some cases especially in *Rhizopogon luteolus* only a slight inhibition was noted. However three strains of bacteria stimulated growth of this fungus at pH 5.0. It appears from the results presented in this work (Figs. 1—4) that in 7-day-old cultures of *Suillus luteus* and *S. bovinus* inoculated with bacteria, growth stimulation of *S. bovinus* and *S. luteus* was noted at all pH levels. However a somewhat smaller enhancement of growth was observed in *S. bovinus* than *S. luteus*.

Seven-day-old cultures of *Amanita muscaria* inoculated with bacteria were less stimulated than *S. bovinus* and *S. luteus*. Also a more pronounced stimulation was observed at pH 5.0 and 6.0 than at pH 4.0 in *A. muscaria*.



Remarks: A = Fungus and bacteria inoculated simultaneously
B = Bacteria introduced into 7 days old cultures of the fungus

Figure 1: Growth of *Amanita muscari* (L. ex Fr.) O. Kuntze

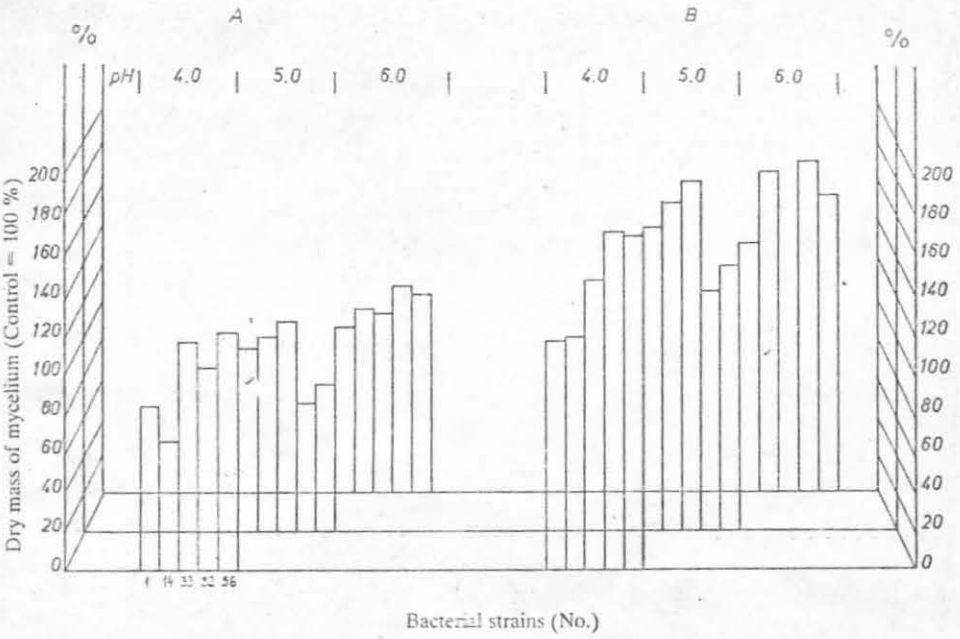


Figure 2: Growth of *Suillus bovinus* (L. ex Fr.) O Kuntze in associative cultures with bacteria

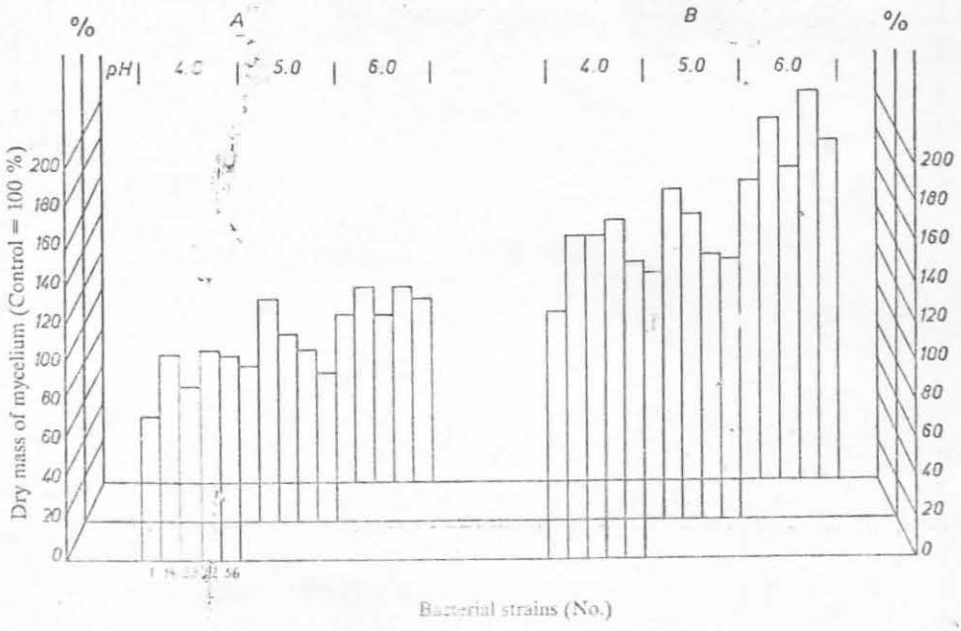


Figure 3: Growth of *Suillus luteus* (L. ex Fr.) S. F. Gray in associative cultures with bacteria

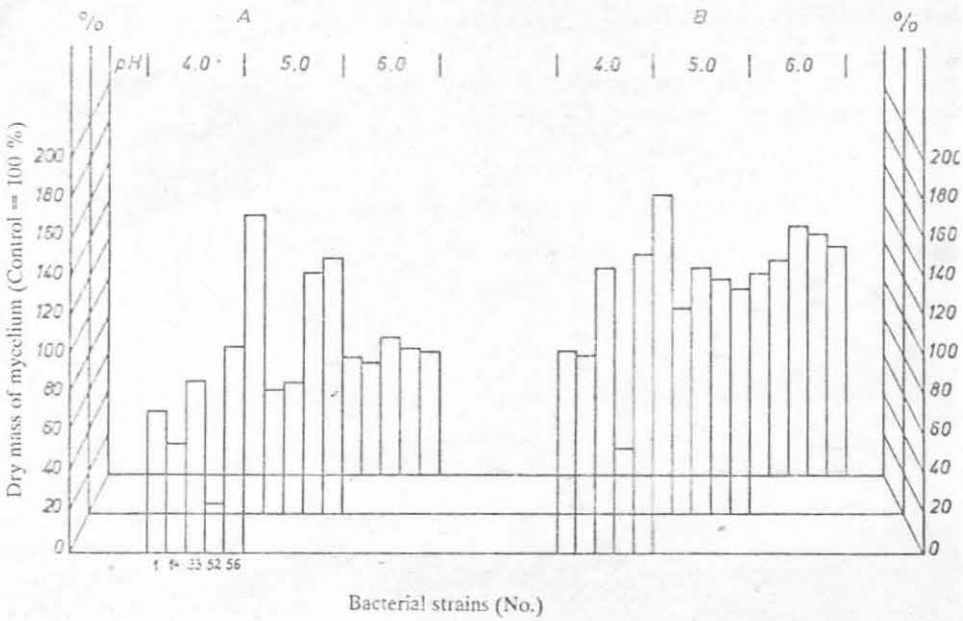


Figure 4: Growth of *Rhizopogon luteolus* Fr. et Nordh in associative cultures with bacteria

In one case the bacterial strain No 52 inhibited growth of *A. muscaria* in cultures inoculated simultaneously as well as in those where the bacteria were introduced into 7-day-old cultures of the fungus only at pH 4.0. However at pH 5.0 and 6.0 a significant stimulation of this fungus by all bacteria was observed.

Regardless of the pH value, growth of *R. luteolus* was less enhanced than other fungi when bacteria were introduced into 7-day-old cultures of fungi. Only one strain No 1 stimulated growth of this fungus with either the simultaneous or delayed bacterial inoculation.

Discussion

The causes of the inhibition of the fungi in mixed cultures could be either the harmful action of bacterial metabolites or competition for nutrients. Enhancement of growth may have been due to the stimulatory effect of bacterial metabolites. Antibiosis, competition and enzymatic action are suggested as mechanisms responsible for depression of fungi in soil (Wright, 1956; Mitchell and Alexander, 1963; Baker and Cook, 1974).

However soil microorganisms may exert no effect or even stimulate growth (and spore germination) of soil pathogens and mycorrhizal fungi (Bowen and Theodorou, 1979; Strzelczyk et al., 1985). Organic substances excreted by the roots of plants and associated microorganisms may affect saprophytic and symbiotic microorganisms living in the root zone. Influence of plant hormones on germination of conidia of *Fusarium culmorum* was reported by Michniewicz and Rożej (1984). Such hor-

mones as auxins, gibberellins and cytokinins are widely produced by microorganisms (Kampert and Strzelczyk, 1984; Strzelczyk and Pokojska-Burdziej, 1984).

Vitamins are essential for many mycorrhizal and pathogenic fungi (Moser, 1959; Davey, 1971; Palmer, 1971; Slankis, 1973). Growth of Basidiomycetes is stimulated by thiamin and in fewer cases by biotin (Norkrans, 1950).

According to Moser (1959) the main source of vitamins for the mycorrhizal fungi are likely to be the rhizosphere microorganisms rather than the plant itself. Therefore in the studies on mycorrhizae forming fungi the accompanying rhizosphere microorganisms should be considered. This however is not a common practice as yet.

Harley (1948) pointed out that saprophytic soil microorganisms may affect mycorrhizae formation. Therefore mycorrhizal studies should be done in conjunction with research on microorganisms associated with mycorrhizae. Many non-symbiotic bacteria and fungi may promote mycorrhizae formation. Such effects are ascribed to metabolites and among them to the vitamins they produce (Shemakhanova, 1965; Vendenyapina, 1967; Slankis, 1973) and amino acids (Melin, 1953; Peller and Mullins, 1982). A review on this topic was published by Rambelli (1973).

Further research on the effect of microbial metabolites on growth of mycorrhizal fungi and mycorrhizae formation is required.

Zusammenfassung

Gemeinsames Wachstum von Mykorrhizapilzen und Bakterien

Kulturen von vier verschiedenen Mykorrhizapilzen der Kiefer (*Pinus silvestris* L.) wuchsen bei 20 °C und pH 4, 5, oder 6 mit jeweils fünf verschiedenen Bakterienstämmen, die aus der Mykorrhizosphäre der Kiefer isoliert wurden. In Medien, die gleichzeitig mit dem Pilz und dem Bakterium beimpft wurden, war das Wachstum des Pilzes in den meisten Fällen leicht stimuliert.

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